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RESEARCH ARTICLE

The Effects Of Melatonin Treatment On Some Serum Immunoregulatory Cytokine Levels In Rats Exposed To Chronic Cadmium Toxicity

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ABSTRACT

The present study aimed to investigate the effect of melatonin treatment on some serum immunoregulatory cytokine levels in rats exposed to chronic cadmium toxicity. For this purpose, animals (n = 32) were divided randomly into four equal groups as untreated control (C), cadmium (Cd), melatonin (Mlt) and Cd + Mlt (CdMlt). The rats in Cd and CdMlt groups received cadmium chloride ($CdCl_2$) (2 mg/kg/day) orally by gastric gavage three times a week for 4 weeks. On the other hand, Mlt (100 mg/kg/day) was orally administrated to Mlt and CdMlt groups five times a week for 4 weeks. C group was not received any treatment. After the treatments, the animals were sacrificed and blood samples were taken to without anticoagulant tubes. Then, levels of IL-1β, IL-2, IL- 6, TNF-α, and INFγ in the serum were determined. It was not found any change among the groups according to IL-1β, IL-2, and IL-6 levels ($p>0,05$). Besides, the administration of Mlt ameliorated the TNF-α levels in CdMlt group compared to Cd ($p<0,05$). IFNγ levels were found the highest in C and Mlt groups compared to Cd ($p<0,05$). In conclusion, Mlt treatment caused a significant change only in TNF-α levels in rats exposed to Cd.
Keywords: Cadmium, Cytokine, Melatonin, Serum, Rat

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Melatonin Uygulamasının Kronik Kadmiyum Toksikasyonuna Maruz Kalan Sıçanlarda Bazı Serum İmmun-Regülatör Sitokinler Üzerine Etkisi

ÖZ

Bu çalışmada kronik kadmiyum toksikasyonuna maruz kalan sıçanlarda melatonin'in bazı serum immun-regülatör sitokin seviyeleri üzerine etkilerinin araştırılması amaçlanmıştır. Bu amaç doğrultusunda, hayvanlar (n =32); kontrol grubu (K) kadmiyum grubu (Cd), melatonin grubu (Mlt) ve kadmiyum + melatonin grubu (CdMlt) olmak üzere rastgele dört eşit gruba ayrıldı. Cd ve CdMlt gruplarındaki hayvanlara, 4 hafta boyunca haftada üç kez gastrik gavaj yoluyla oral kadmiyum klorür ($CdCl_2$) (2 mg/kg/gün) verildi. Öte yandan Mlt ve CdMlt gruplarına 4 hafta boyunca haftada beş kez oral Mlt (100 mg/ kg/gün) uygulaması yapıldı. Kontrol grubunda yer alan sıçanlara herhangi bir uygulama yapılmadı. Deney periyodundan sonra, sıçanlar sakrifiye edildi ve kan örnekleri antikoagulant içermeyen tüplere alındı. Deneme sonunda serum IL-1β, IL-2, IL-6, TNF-α ve INFγ konsantrasyonları belirlendi. IL-1β, IL-2 ve IL-6 seviyelerinde deney grupları arasında bir değişiklik bulunmadı ($p>0,05$). Ayrıca, Mlt uygulaması CdMlt grubundaki TNF-α düzeylerini Cd grubuna kıyasla iyileştirdi ($p<0,05$). IFNγ seviyeleri Cd grubu ile karşılaştırıldığında en yüksek oranda C ve Mlt gruplarında tespit edildi ($p<0,05$). Sonuç olarak, Mlt uygulaması Cd toksisitesine maruz kalan sıçanlarda sadece TNF-α düzeylerinde önemli bir değişikliğe neden olmuştur.
Anahtar Kelimeler: Kadmiyum, Melatonin, Serum, Sitokin, Sıçan

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INTRODUCTION

Cadmium (Cd) is, a toxic heavy metal, a worldwide significant environmental pollutant that exerts a variety of adverse effects on humans and also animal health (Dukic-Cosic et al. 2020). It is used in many industrial areas include the nickel-Cd batteries, sensors, televisions, metal-electro plating, pigments, plastics, and alloy (Klassen et al. 2009, Predes et al. 2010). Although living beings are mainly exposed to Cd through the intake of contaminated water, food, plants (vegetables) or air, dermal exposure (through the skin) is uncommon (IARC 2012, Kisadere and Donmez 2019). It has been reported that long-term Cd accumulation causes serious tissue damages in many organs and biological systems (Cuypers et al. 2010). Although Cd causes oxidative stress by increasing lipid peroxidation (LPO) and/or by changing intracellular glutathione (GSH) levels, recent studies have shown that it can also affect the immune system functions in humans and animals (Patra et al. 2011, Kisadere et al. 2019, Turley et al. 2019). Marth et al. (2001) have been reported that at low concentrations, Cd is able to stimulate the immune system, while at higher concentrations it has inhibitory and immune-suppressive properties.

Inflammation is a very complex process, a preventive response of the organism to injury (physical, chemical, etc.) or infections. In a part of this process, different types of immune cells (macrophages, B/T lymphocytes, mast cells, and endothelial cells, etc.) produce biochemical compounds of inflammatory and immune reactions called cytokines. They have a specific effect on the interactions (synergistically or antagonistically) and communications between cells (Zhang and An 2007). They are divided into two groups as pro-inflammatory and anti-inflammatory cytokines. Excessive secretion of these mediators (~5–20 kDa), however, cause functional problems in different type of immune cells (Moniuszko-Jakoniuk et al. 2009). One of them is interleukin-1β (IL-1β), a pro-inflammatory cytokine, is crucial for host-defense system responses to infection and injury (Dinarello 1997). Also, IL-2 plays a major role in the activation, proliferation, and differentiation of T, B lymphocytes and natural killer (NK) cells. Another important multifunctional pro-inflammatory cytokine is IL-6 that plays critical roles in host defense, acute phase response, B cell proliferation, and thrombopoiesis (Hirano 1998, Zhang and An 2007). An increased serum IL-6 values have been related to various pathological conditions, including infections, physical trauma, inflammations, auto-immune problems and different types of malignancies (Taga and Kishimoto 1997). Tumor necrosis factor-alpha (TNF-α) also plays major roles in microbial infections, cell death, inflammation, pain, and the growth of different malignant tumors. Also, elevated TNF-α levels have been implicated with some serious problems such as cachexia, septic shock, and autoimmune diseases (Ware et al. 1996). Interferon gamma (IFNγ) is, type two (II) interferon, a critical for innate and adaptive immunity of host against various viral, bacterial, and protozoal infections. It also has immunostimulatory and immunomodulatory effects include: induces of class I MHC and class II MHC in different cells, activates macrophages, neutrophils, natural killer cells (NK), promotes cell-mediated immunity (Zhang and An 2007). Szuster-Ciesielska et al. (2000) have been announced that some cytokines include TNF-α, IFNγ and IL-6 may be influenced by the exposure to Cd.

In recent years, it has been reported that the detrimental effects of Cd can be alleviated by using some substances that have antioxidant and metal-binding properties. Many chelating agents and antioxidants have been used to diminish tissue damages in chronic Cd exposed animals (Pourmorad et al. 2006, Karabulut-Bulan et al. 2008). One of them is Mlt (n-acetyl-5-methoxytryptamine), a neuro-hormone, was secreted mainly from the pineal gland. The control of biologic circadian rhythms, sleep pattern-induction, regulation of seasonal reproduction, food intake, and immune enhancement can be described as a biological function of Mlt (Maestroni 1998). It has been suggested that it is a powerful antioxidant and free radical consumer substance due to its small size and lipophilic properties. Besides, it has a metal binding function (Karbownik et al. 2001, Dan et al. 2018). In addition, recent studies have shown that Mlt (an immune modulator) has regulatory effects on immunity and anti-inflammation (Shin et al. 2014). On the other hand, Kim et al. (2000) have been reported that Mlt restores significantly the immunotoxic status induced by Cd in mice. Also, positive immuno-regulatory effects of Mlt on different heavy metal intoxications were reported by different researchers (Bali et al. 2016, Li et al. 2016, Dutta et al. 2018, Durappanavar et al. 2019).

The purpose of this study was to determine the effects of oral Mlt treatment on some serum immunoregulatory cytokine levels in rats exposed to low dose chronic Cd toxicity.

MATERIALS and METHODS

Animals and Experimental Design

Male albino Wistar rats (n = 32; 3 weeks old; body weight ~ 200 ± 30g) were obtained from Balıkesir University Experimental Medicine Research and Application Center (BUEMRAC). After one week of acclimatization period, animals were divided randomly into four groups as detailed below: untreated control (C), cadmium (Cd), melatonin (Mlt) and Cd + Mlt (CdMlt) groups; each group contained 8 animals. Animals were, housed in standard plastic rat cages (polypropylene), maintained in an air-conditioned room (BUEMRAC; temperature: 23 ± 2 C^0; humidity: 55 ± 10%) on a 12-h light/dark cycle

with fresh-water and food available ad libitum. The animals in Cd and CdMlt groups received cadmium chloride ($CdCl_2$) (2mg /kg/ day) orally by gastric gavage three times a week for 4 weeks (Almenara et al. 2013). On the other hand, Mlt (100 mg/kg/day) was orally by gastric gavage administrated to Mlt and CdMlt groups five times a week for 4 weeks (Haddadi et al. 2015). At the end of the 4-weeks experiment period, rats were sacrificed under anesthesia using an intramuscular injection of ketamine/xylazine (0.1 ml/100gm/body weight). Blood samples were drawn by cardiac puncture using without anticoagulant tubes. Then, they were centrifuged (at 3000 g for 20 min) after coagulation and serum were separated. The serum samples not used immediately were frozen at -80ºC until further analysis. The levels of IL-1β, IL-2, IL-6, TNF-α, and INFγ in the serum were determined.

Determination of Some Serum Cytokine Levels

The levels of IL-1β, IL-2, IL-6, TNF-α, and IFNγ in the serum were measured by enzyme immunoassay using ELISA kits from Sunred Biological Technology (Shanghai, China) according to the kit instruction. This assay based on a double-antibody sandwich ELISA assay to measure the levels of rat IL-1β, IL-2, IL-6, TNF-α, and IFNγ in the serum. In brief, serum samples and standards provided in the kit were extracted on an extraction plate, derivatized using an equalizing reagent, and subjected to ELISA in IL-1β, IL-2, IL-6, TNF-α, and IFNγ pre-coated microtiter strips. The absorbance of the solution in the wells was read at 450 nm within 15 min using a microplate reader (Thermo Scientific Multiskan FC, USA). The optical density was used to calculate the cytokine levels using a standard curve.

Statistical Analysis

The statistical analysis of the data was done using by analysis of variance (one way-ANOVA) followed by Duncan's test using the SPSS 25.0 package program (SPSS, Inc., Chicago, IL). Value for $P \leq 0.05$ were considered as statistically significant.

RESULTS and DISCUSSION

The results of the study were shown in Table 1. It was not found any significant change among the experimental groups according to IL-1β, IL-2, and IL-6 levels ($p>0,05$). On the other hand, serum TNF-α levels were detected the highest in the Cd group when compared to other experimental groups ($p<0,05$). Besides, the administration of Mlt ameliorated the TNF-α levels in CdMlt group compared to Cd ($p<0,05$). In addition, IFNγ levels were found the highest in C and Mlt groups when compared to Cd and CdMlt ($p<0,05$).

In the present study, Cd (2 mg/kg/p.o for 4 weeks) administration did not lead to any significant change in serum IL-1β, IL-2, and IL-6 levels in all experimental groups. Afolabi et al. (2012) reported that different doses of Cd (50-100 ppm/p.o for 7 weeks) treatment led to an increase in plasma IL-2 and IL-6 levels in rats. Also, Moniuszko-Jakoniuk et al. (2009) suggested that the levels of IL-6 elevated in rat serum only after oral treatment of Cd (50 mg/kg) for 16 weeks. In a previous study, Yücesoy et al. (1997) announced that long term (one year) exposure did not alter serum IL-2 levels in the factory workers who were directly exposed to Cd. It might be changed due to different dose only. On the other hand, Mlt (100 mg/kg) treatment did not cause any significant change in the levels of IL-1β, IL-2, and IL-6 in our study. It was not found any information about the effects of Mlt on serum IL-1β, IL-2, and IL-6 levels in rats exposed to chronic Cd in the literature. Bali et al. (2016) announced that Mlt (10 mg/kg/bw p.o) administration significantly decreased the serum levels of IL-6 in arsenic (As)-induced liver damaged rats. Also, Durappanavar et al. (2019) reported that per-oral Mlt administration (10 mg/kg/bw) suppressed the release of IL-1β, IL-6, and TNF-α in the brain tissue of Wistar rats that were exposed to As.

In our study, serum TNF-α levels were determined the highest in Cd group compared to C, CdMlt, and Mlt. Alghasham et al. (2013) suggested that Cd (40 mg $CdCl_2$/L) treatment significantly increased the plasma levels of TNF-α and IL-6 in rats exposed to Cd-polluted water for six (6) weeks. Moniuszko-Jakoniuk et al. (2009) also informed that levels of some main proinflammatory cytokines were remarkably increased in the serum of experimental animals after long term exposure to Cd. Also, another researchers have noticed that accumulation of Cd induces the production of TNF-α and IL-6 in some living beings (Kataranovski et al. 1998, Krocova et al. 2000). On the contrary, Yücesoy et al. (1997) noticed that long-term (one year) Cd administration (average 1.8-25.3 μg/l) did not alter serum TNF-α levels in the workers of battery production company. The differences could be occurred due to the chemical form of the heavy metal, application route, dosage regime, exposure time, and genetic properties of host. In the present study, Mlt treatment ameliorated the serum TNF-α levels in CdMlt group compared to Cd. It was not found any significant information about the effects of Mlt on serum TNF-α levels in rats exposed to chronic Cd in the literature. Besides, Dutta et al. (2018) reported that Mlt treatment reduced the levels of both TNF-α and matrix metalloproteinase-2 (MMP_2) in As intoxicated kidney injury. Our results were also corresponding with the previous studies (Li et al. 2016, Durappanavar et al. 2019).

In our study, IFNγ levels were found the lowest in Cd and CdMlt groups compared to C. Theocharis et al. (1991) also detected a decrease in IL-2 and IFNγ levels in the presence of 10^{-4} M Cd^{2+}. Besides, Szuster-Ciesielska et al. (2000) suggested that Cd

effect depended on the concentration used, and 1 and 10 μM CdCl2 partially, but 100 μM Cd completely inhibited the production of TNF-α and IFNγ in bovine aorta endothelial cells. On the other hand, Moniuszko-Jakoniuk et al. (2009) reported that exposure to Cd in both 5 and 50 mg Cd/l doses, alone or in combination with ethanol (EtOH), led to an increase in the serum levels of IL-1α, TNF-α, and INFγ in rats. Similarly, Yücesoy et al. (1997) detected an increase in the serum IFNγ levels in the long-term low-dose Cd-exposed workers. In the present study, low dose (100mg/kg for 4 weeks) oral Mlt treatment did not attenuate the IFNγ levels in CdMlt group compared to C. Although Mlt has anti-inflammatory effects, previous studies about the effects of Mlt on cytokine levels are sometimes contradictory. Srinivasan et al. (2005) have been reported that Mlt increases IL-2, IL-6, IL-12, and IFNγ levels by stimulating cytokine production in aged individuals and patients in an immunocompromised state. In the contrary, Broncel et al. (2007) have been suggested that Mlt decreases IL-6, IL-12, TNF-α, and IFNγ levels in patients who at risk of atherosclerosis. These different results may be occurred due to Mlt could not exhibit its metal-binding properties in that dose.

Table 1. Some serum immunoregulatory cytokine levels of experimental groups.

Parameters	C	Mlt	CdMlt	Cd
IL-1β (pg/L)	782,00±153,36	824,50±23,31	1280,58±330,59	1385,75±365,48
IL-2 (ng/L)	2,82±1,18	2,96±0,26	3,42±0,43	3,68±0,56
IL-6 (ng/mL)	32,63±0,45	32,69±0,61	32,77±0,44	33,41±0,27
TNF-α (ng/L)	576,60±2,33b	576,96±0,91b	578,75±2,06b	586,42±2,33a
IFNγ (ng/L)	61,81±5,10a	56,95±4,05a	40,28±4,92b	39,31±3,43b

Groups: C, control; Mlt, melatonin; Cd, cadmium; CdMlt, Mlt + Cd. [a–b]Means in the same line with different superscripts differ significantly ($p < 0.05$).

CONCLUSION

In conclusion, Mlt (100 mg/kg/day) treatment exhibited a very partial change in some serum immunoregulatory cytokine levels in rats exposed to chronic low dose Cd toxicity. Therefore, further investigations are required for the clarification of these important interactions.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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